Bioaugmentation: From the Start to the State-of-the-Art

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Talk Outline

- Definition
- Factors that Affect Bioaugmentation
- When and Where to Bioaugment
- Some History of the Development of Bioaugmentation
- Some Early Examples
- The Path to Today
- A Few Case Histories
- The Current State of the Art
- Remaining Issues
- Costs

Definition of Bioaugmentation

Bioaugmentation is the addition of a prepared culture of microorganisms with desired degradative properties to a contaminated medium to exploit the degradative qualities to enhance contaminant biotransformation

Factors that Affect Bioaugmentation

- Hydrogeologic conditions such as effective porosity, aquifer heterogeneity, and groundwater flow velocities affect the ability to inject and distribute microorganisms, and the ability to contact bugs, substrate, and contaminant
- Geochemical parameters including pH, salinity, redox (competitive electron acceptors), temperature and contaminant concentration (toxicity concerns) and bioavailability affect the activity and survival of the injected cultures
- Geomicrobiology including competition for electron donor and predation affects the survival of the injected culture
- Substrate Interactions such as with co-contaminants can inhibit degradations rates in complex mixtures

When/Where to Bioaugment

Bioaugmentation is appropriate at sites where:

- Natural attenuation processes are not evident and/or not protective of sensitive receptors
  - Lack of contaminant degraders
  - Lack of nutrients (i.e., primary substrates, suitable electron donors, other nutrients)
  - Poor environmental conditions (i.e., redox, pH)
  - Slow kinetics

  Screened by thorough site characterization and monitoring

- Enhanced bioremediation does not work
  - Lack of microorganisms

  Screened through laboratory and field testing (RABITT)

- Hydrogeologic and geochemical parameters allow for the introduction/distribution of the organisms, delivery or nutrient solutions, and expression/retention of degradative activity

A Little History: In the Beginning

- Bioaugmentation products were based more on profits motivations than on sound science
  - Known that microorganisms degraded contaminants
  - Not understood that specific microorganisms were responsible for that degradation
  - Not understood which environmental variables affected activity and survival
  - Not clear what benefit bioaugmentation provided
- Aggressive sales tactics of “snake oil” salesman led resulted in black eye for bioaugmentation
  - Unsubstantiated claims
  - Failed applications

Some Early Quotes From Vendors

- When asked for application directions

  "Just mix in about 25% and wait. If it doesn't work, just add some more"

- When asked about the degradation pathway

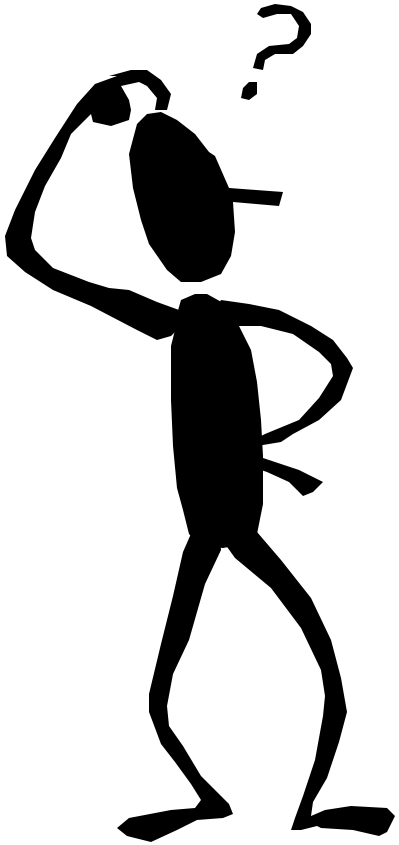
  "The contaminant causes a mutation that enables the bacteria to degrade the contaminant"

- When asked about culture survivability

  "The bacteria don't grow, they're born dead"

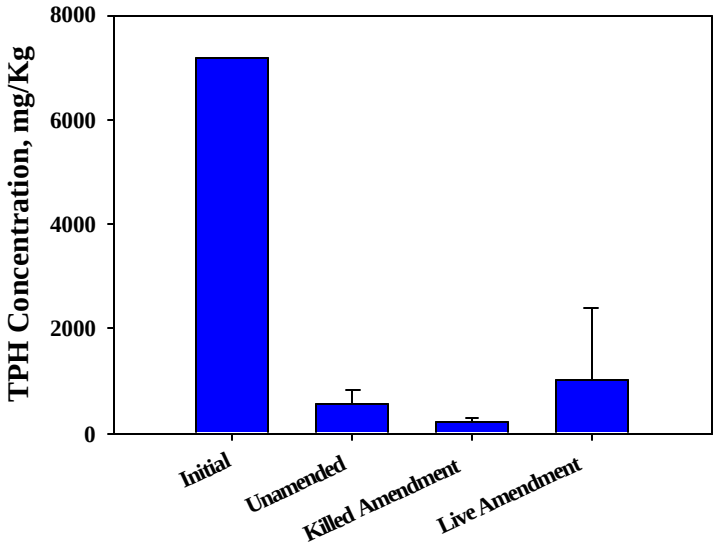
- When asked about oxygen requirements

  "Bacteria like microbubbles, large bubbles are like you trying to swallow a basketball"



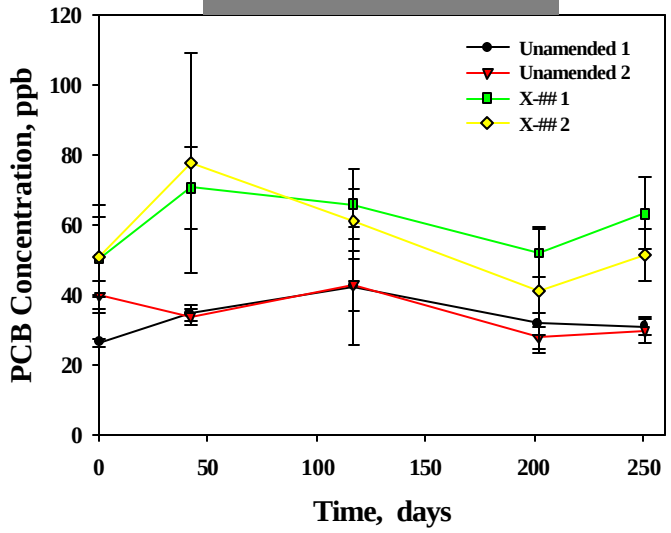
Examples of Early Products

- Product 1: XXX-2000
- Marketed for Petroleum Hydrocarbons
- Claim: faster treatment to lower concentrations
- 3 Part Product
  - Surfactant, nutrient, freeze-dried culture (powder)
- Laboratory tested over 60 days
- Results showed no marked improvement over no amendment



Examples of Early Products

- Product 2: X-XX
- Marketed for hydrocarbons, PAHs, pesticides, PCBs, and metals
- Claims: 500 ppm to ND in 150 days
- 2 Part Product
  - Peat based culture
  - Chemical inducer
- Field tested for 250 days
- Results showed no treatment with or without amendment



The Path Forward

- Involvement of Qualified Scientists and Engineers
  - Microbiologists, geologists, hydrologists, environmental engineers
  - Questions asked, answers sought
- Laboratory Studies
  - Isolation and identification of microorganisms
  - Better understanding of the underlying principles of biodegradation
    - Aerobic, anaerobic, cometabolic pathways
  - Better understanding of the Degradation Environment
    - Redox conditions, pH effects
    - Nutrient requirements
    - Competition and survival
- Field Trials and Demonstrations
  - Microbial Transport
  - Culture Survivability
  - Expression of degradation activity

3 Case Histories

- #1 is and aerobic approach that utilized addition of methane oxidizing bacterium
  - Cometabolize TCE via methane monooxygenase
- #2 involved aerobic degradation of TCE toluene degrading bacterium
  - Cometabolism by a constitutive toluene *ortho*-monooxygenase producing bacterium
- #3 employed a mixed culture of anaerobic halorespiring microorganisms
  - Mixed cultures of phylogenetically related *Dehalococcoides*

Case History #1: Chico Municipal Airport LLNR

Culture

- *Methylosinus trichosporium* OB3b (pure culture in resting state)
- Laboratory grown and concentrated, suspended in TCE-free groundwater

TCE plume

- (2000 m x 500 m; 1.0 to 1.5 ppm max)

Aquifer characteristics

- Depth 26 m, porosity .40, permeability 3μm^2, velocity 30 cm/d

Test Setup

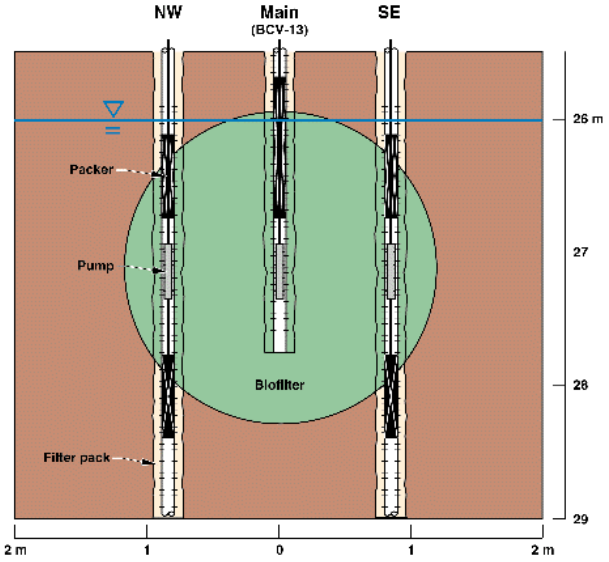
- 1 injection well (IW), 2 monitoring wells (MWs)
- 1,800 L, 5.4 x 109 cell/mL, 1 well, 3.8 L/min
- No substrate added

Water Extraction

- 3.8 L for 30 hours then 2.0 L/min from IW

Sampling and Analysis

- Extracted water and 2 MWs
- TCE and tracer analyses and microbial enumerations



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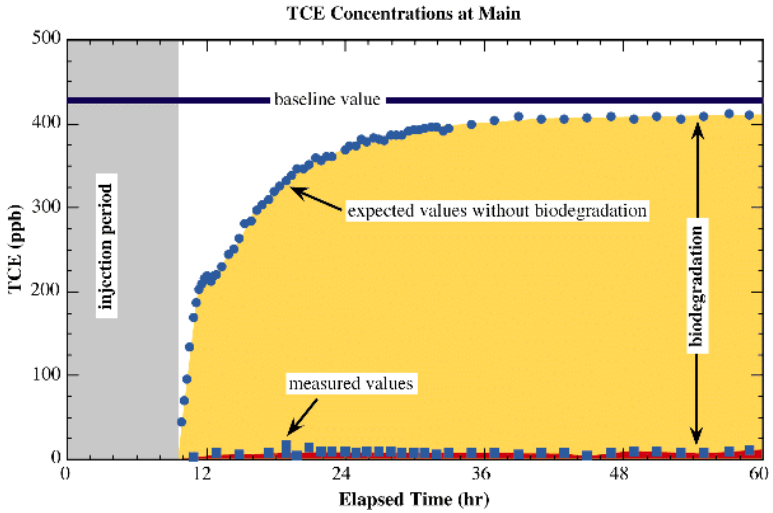
Case History #1 (continued)

Results

- TCE reductions of 98% (425 ppb to less than 10ppb) over the first 50 hours
- TCE concentrations increased to background levels after 40 days
- Approximately 50% of injected cells were recovered in extracted groundwater
- Culture did not survive presumably due to the lack of primary substrate, not predation

Conclusions

- Demonstrated initial level of activity
- Both culture activity and survivability need to be extended to be a viable approach
- Addition of substrate (methane) is required



Case History #2: Industrial Site, NJ Envirogen

Culture
- *Burkholderia cepacia* ENV435 (adhesion deficient variant of *B. cepacia* $PR1_{301}$)
- Laboratory grown on sucrose and phenol, transferred to holding tanks on site

TCE plume
- 1.0 to 2.5 mg/L, TCE, DCE isomers, vinyl chloride

Aquifer characteristics
- Heterogeneous, silt and fine to medium-grade sands, thin clay lenses
- K_h 1.13 – 2.70, $?_e$ 0.16, groundwater velocity 0.89 m/d

Test Setup
- Control plot and test plot, 4.6 m x 12 m, 3 nested IWs, 3 rows of nested MWs, 1 recover well, single MW at end of plot, 1 nested MW between plots
- Two injection modes, extraction and recirculation (550 L at 2.3 – 3.0 L/min to achieve 1 x 10^{11} cells/mL), direct injection into MWs followed by pneumatic flush with pure O_2; BSM solution added to control plot
- No additional substrate added

Sampling and Analysis
- Extracted water from MWs
- Chlorinated solvents, O_2, and bromide tracer analysis
- microbial enumerations via plate counting

Case History #2 (continued)

Results

- 1st Injection
  - VOC concentrations reduced by as much as 77% (~2,220 µg/L to <500 µg/L)
  - Culture distributed across the 12 m plot along preferential flow paths
  - Loss of cells due to filtration and/or death (half life - 1 to 2 days)
- 2nd Injection
  - VOC concentrations were reduced to as low as 50 µg/L

Conclusion – Limited Success

- Limited distribution of culture, suggests biobarrier potential
- Limited retention/survival of culture
- Multiple injections and oxygen constraints are not attractive for full-scale application
- Addition of substrate is required to retain activity (note: attempts with *B. cepacia* with substrate have resulted in excessive clogging)

Case History #3: Kelly AFB, Texas RTDF and ESTCP

Culture
- KB-1 (mixed culture containing phylogenic relatives of *Dehalococcoides ethenogenes*)
- Grown in two 8-L stainless steel pressure vessels on methanol and TCE and purged with 80:20 N2:CO2

TCE plume
- 1.0 mg/L PCE, lesser amounts of TCE and *cis*-DCE

Aquifer characteristics
- Unconsolidated alluvial deposits consisting of gravels, sand, silt and clay
- Groundwater velocity 0.9 m/d

Test Setup
- Sealed bottle microcosm study and pilot-scale field test
- Control plot and test plot, 10 m, I IW, 5 MWs, 3 EWs
- Inoculation with 13 L KB-1
- Recirculation rate of 5.7 L/min.
- 3.6 mM each of methanol and acetate

Sampling and Analysis
- Extracted water from MWs
- Chlorinated solvents, methanol, VFAs, bromide tracer
- microbial analysis via PCR and 16S rDNA

Case History #3: Kelly AFB, Texas

Results

- Microcosm Study
  - Controls – TCE to *cis*-DCE in ~ 20 days
  - Augmented – complete conversion of TCE to ethene by day 150
- Field Test

- Extended Test
  - *Dehalococcoides* persisted for over one year without electron donor addition
  - Reductive dechlorination was held up at *cis*DCE
  - Electron donor addition lead to recovery of complete dechlorination to ethene

Conclusions

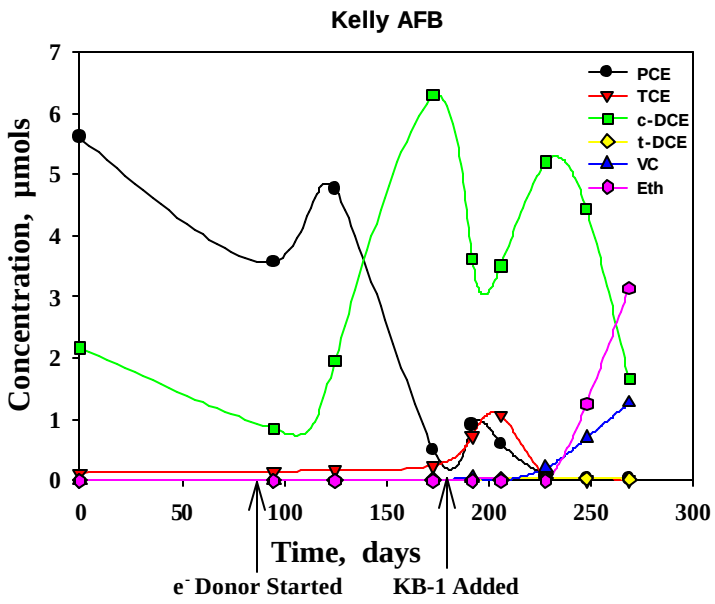
- Demonstrated distribution of culture
- Demonstrated long-term survival
- Demonstrated retention of activity
- Technology selected for full-scale application

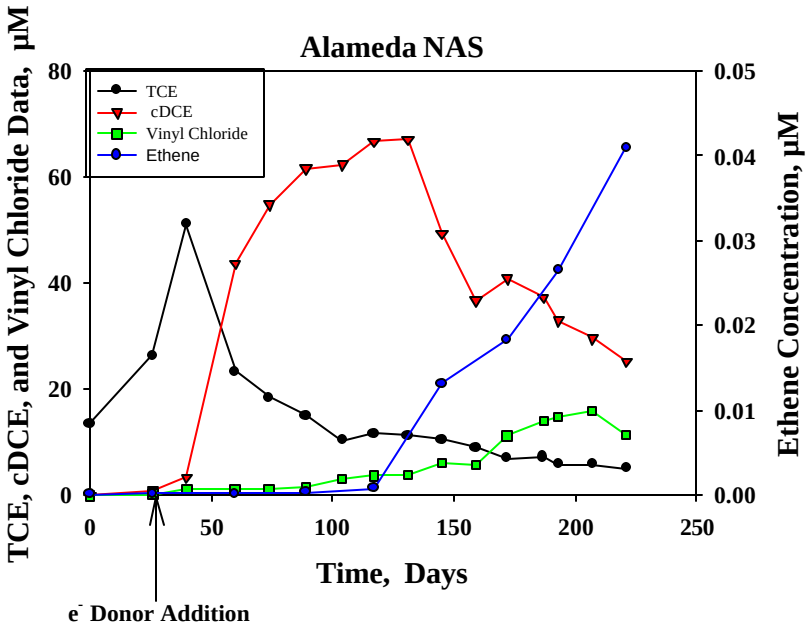
Current State-of-the-Art

- Screen Sites for Need, Ability to Inject/distribute Amendment, and potential for success
- Select appropriate amendment approach
  - Aerobic, cometabolic, anaerobic
- Select proven culture
  - Survivability and retention of activity
- Use sound engineering principles to design the treatment
  - Biobarrier, funnel & gate, etc…
  - Based on contaminant distribution and aquifer properties
- Bioaugment using proven inoculation procedures
  - Direct injection, pneumatic injection
- O&M similar to enhanced bioremediation

Is Bioaugmentation Really Necessary?

(Suthersan, S.S., 2001. *Natural and Enhanced Remediation Systems*. Boca Raton: Lewis Publishers)





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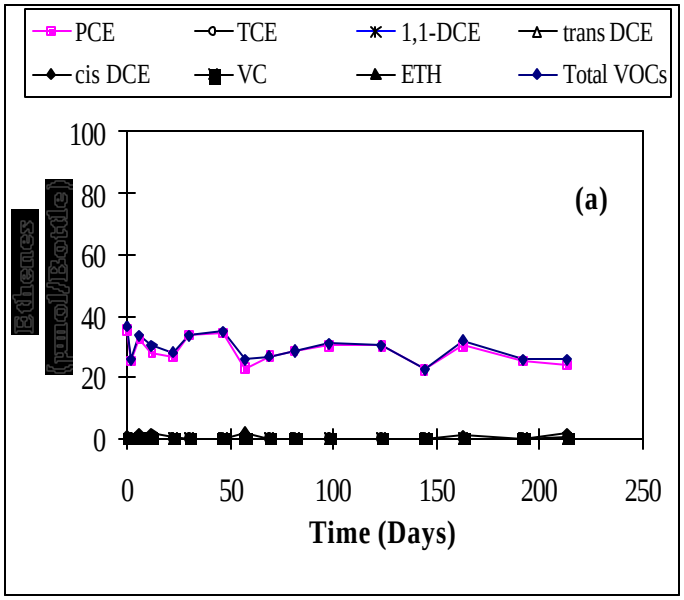
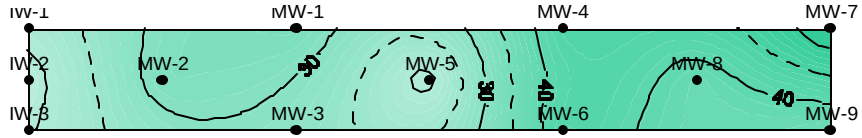
Use of Microcosms as the Predictor?

RABITT at Camp Lejeune



Day 0

PCE (µM)



Day 196

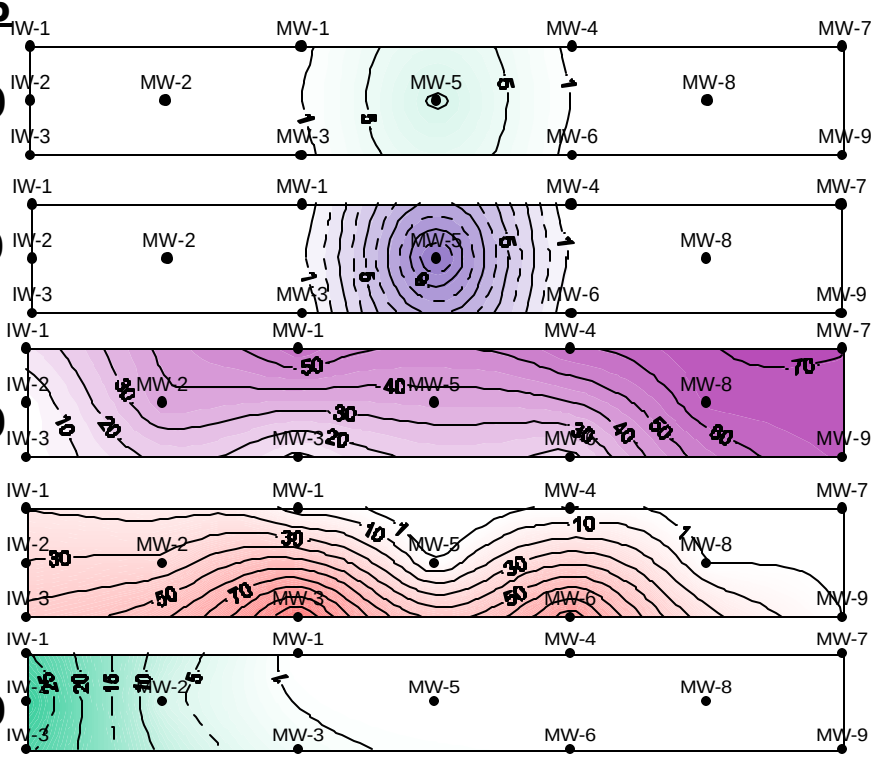
PCE (µM)

TCE (µM)

DCE (µM)

VC (µM)

ETH (µM)



Promoting Readiness through Environmental Stewardship

Cost Estimate: Biobarrier, 200 ft x 1,000 x 10 ft plume, 25 ft depth to water (ESTCP Cost&Performance)		
Cost Category	**Subcategory**	**Costs ($)**
	FIXED COSTS	
1. CAPITAL COSTS	**Work Plan**	**$65,000**
	Microcosm Testing	**$84,500**
	Site work, (Well Installation, Trenching)	**$71,500**
	Equipment (Pumps, Piping, Mixing/Delivery Equipment	**$21,0000**
	Microbial Culture	**$25,000**
	Installation	**$21,000**
		Subtotal $288,000
	VARIABLE COSTS	
2. OPERATION AND MAINTENANCE	**Labor (Operation and Maintenance (Annual))**	**$72,000**
	Materials and Consumables	**$8,000**
	Travel costs	**$20,000**
	Chemical/Biological Analyses	**$14,420**
	Performance Data Analysis/Reporting	**$11,454**
	Trailer Rental	**$9,600**
		Subtotal $135,474
		TOTAL TECHNOLOGY COST : $423,474